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Original paper

Possible detection of physiological state disorders with the help of medicinal leeches *Hirudo verbana* Carena, 1820

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ABSTRACT. In the experiment, 160 medicinal leeches of the species *Hirudo verbana* Carena, 1820 were studied. Medicinal leeches were fed on the blood of animals and people (conditionally healthy and diseased). Four leeches were taken from each animal/person. The animals were studied for 3 weeks. Mortality was mostly observed in the first days after feeding on the blood of the host. We noted mortality, the appearance of constrictions on the leeches' body, the intensity of the host blood spitting from their body. The host's blood was taken from their stomach on the first day after feeding. Hematological and immunological indicators of blood were determined in the taken blood of the host. As a result of the study of the blood of the sick, significant changes were found, compared to conditionally healthy ones. It was manifested by an increase in erythrocytes and leukocytes. The leukocyte formula looked like in most pathological conditions of the inflammatory process. The obtained indicators of the experiment make it possible to quickly assess the presence of physiological disorders in the early stages of the disease.

Keywords: medicinal leech, blood, physiological state, organism

Introduction

Hirudotherapy is a method of alternative medicine in which medicinal leeches are used for therapeutic purposes [1]. According to scientific data, this method is currently used in the prevention and treatment of many infectious and non-infectious diseases [2–11] due to the presence of a significant amount of biologically active substances in her body, which are able to show various therapeutic effects [1–13]. New effects of it are discovered every year. For example, it was recently experimentally proven that the substances of the medicinal leech are able to neutralize *Mycobacterium tuberculosis* [14]. The study revealed that the medicinal leeches are rich in oleic acid (33.9%) and palmitic acid (22.6%). The anti-tubercular activity of the medicinal leeches was attributed to the high oleic and palmitic acid [14]. Medicinal leeches neutralize cancer cells and reduce pain [15,16], restore lungs after Covid-19 [17], modulate various inflammatory processes, accelerate wound healing, etc. [1,18–22].

It should be noted that the growth of various pathological conditions in the world has led not only to the intensive search for various methods of their treatment, but also to the search for methods of their rapid diagnosis. A large number of all diseases, if untimely detected, can cause irreversible changes in the body or even death. According to the results of other scientists and our previous studies after hirudotherapy changes in various hematological and immunological parameters of the host's blood [18–20]; on 9–14 days after hirudotherapy in 9.5±0.57% medicinal leeches pathological manifestations of morphophysiological features were observed, which resulted in the death of 4.5±0.48%. Morphofunctional changes in the midgut of a well-fed healthy *H. verbana* 12 days after feeding on human blood change the height of the epithelium and the level of its vacuolization, and in a well-fed leeches that spit blood and die, these

changes are accompanied by degenerative processes [23]. According to these studies, medicinal leeches could hypothetically die as a result of eating the blood of sick people, which has not been previously paid attention to medicinal leeches and the blood they consumed from the side of express diagnostics outside the body of the host.

Therefore, the study of the condition of medicinal leeches and the analysis of the host's blood eaten by them became the relevance and purpose of this study in order to develop rapid diagnostics to detect any violations of the host's physiological state.

Materials and Methods

Group of animals

A total of 160 medicinal leeches of the species *Hirudo verbana* Carena, 1820 were used in the experiment. Two studies were conducted. The first experiment was a study of 80 medicinal leeches that were fed once on the blood of a sexually mature person (4 leeches from each person). Totally, 40 medicinal leeches were used, which were fed on the blood of a conditionally healthy person, who did not consult a doctor, the general condition was normal, the general blood parameters were normal (the first group, control). In the second group, 40 medicinal leeches were also used, which were fed on the blood of sick people (the diagnosis was confirmed in the hospital) during the manifestation of various diseases: varicose veins, arthrosis and osteochondrosis, angina pectoris, disorders of the gastrointestinal tract, initial stages of prostatitis etc. In the second study, medicinal leeches were used, which were fed once with the blood of non-linear sexually mature laboratory rats. Totally, 80 medicinal leeches (4 leeches from each animal) fed on the blood of non-linear laboratory rats were studied. In first group of 40 medicinal leeches fed on the blood of conditionally healthy animals all hematological and immunological parameters of the blood corresponded to the physiological norms of the given age. The second group of 40 medicinal leeches were fed on the blood of sick animals: ulcerative dermatitis, suppuration of wounds in various parts of the body, burns, diseases of the respiratory organs, abscesses, etc.

Experiment scheme

Medicinal leeches were taken, which fed no more than a day on the blood of a person or an animal (conditionally healthy and with pathology). According to known facts, blood eaten by a leech does not change its physiological composition during this time. For three weeks, their general condition was observed: death (with a changed body shape and without changes), the appearance of stretch marks on their body, intense spitting of eaten blood. It should be noted that death was noted in the majority in the first days after feeding. With the help of tweezers, the host's blood was neatly squeezed out of their body into a test tube. Hematological and immunological indicators of eaten blood with the addition of 2% heparin solution („Spofa") were studied. Blood parameters of conditionally healthy and sick were compared.

Statistical analysis

Statistical data processing was performed using the computer program SPSS v.23,0. (IBM SPSS Statistics., USA). The selected parameters indicated in the table below have the following notation: X – the average value of the sample, SE – standard error of the average value of the sample. The significance of differences between the mean values was evaluated by the Student's criterion after checking the normal distribution. Differences were considered significant at $P<0.05$.

Bioethics

Animal manipulation was carried out in accordance with the rules and regulations for the treatment of laboratory animals: principles of bioethics, legislation, and requirements in accordance with the provisions of the „European Convention for the Protection of Vertebrate Animals Used for Research and Scientific Purposes", the Law of Ukraine „On the Protection of Animals from Animals Handling".

Results

As a result of the research in the first experiment (human blood) in pathological conditions, we observe an increased death of medicinal leeches, the appearance of constrictions on their bodies, and the intensity of spitting out the eaten blood is more than 2 times (Tab. 1). When analyzing hematological and immunological indicators of blood, they do not correspond to the physiological norms. The total number of erythrocytes increased by 30.8±2.9%, leukocytes by 35.6±3.1%, compared to the control. In the blood leukocyte formula of sick people,

Table 1. General condition of medicinal leeches, red blood cells, number of leukocytes and blood leukocyte formula of eaten human and animal blood, X±SE

Studied material		Medicinal leeches (n=160)			Eaten human (n=20) and animal blood (n=20)						
		Mortality, %	Narrowing on the body, %	Intensity of blood spitting %	Erythrocytes/l ($\times 10^{12}$)	Leucocytes/l ($\times 10^{9}$)	Lymphocytes, %	Band neutrophils, %	Segmented neutrophils, %	Eosinophils, %	Monocytes, %
Human blood	Conditionally healthy	2.1±0.3	1.5±0.2	3.1±0.1	4.5±0.4	6.5±0.5	23.5±1.2	4.3±0.3	67.2±3.1	2.0±0.5	3.0±0.3
	Sick	5.1±0.4*	3.2±0.4*	7.2±0.2*	6.5±0.3*	10.1±1.1*	35.0±3.5*	15.2±1.6*	30.5±2.1*	3.1±0.6	6.1±0.5*
Animal blood	Conditionally healthy	1.5±0.3	1.4±0.1	2.8±0.3*	5.5±0.3	10.5±0.9	57.8±1.9	0.7±0.02	25.0±1.4	2.5±0.3	3.5±0.5
	Sick	5.7±0.2*	4.5±0.4*	6.6±0.2*	7.0±0.4*	16.2±1.3*	35.0±2.1*	8.0±0.4	33.2±1.2	5.1±0.8*	2.5±0.5

Explanation: * – $P<0.05$ compared to the control group

changes that indicate the manifestation of a pathological condition in the body: the percentage of lymphocytes increased by 32.9±2.9%, the percentage of segmented neutrophils decreased by 45.4±4.3%, the percentage of band neutrophils increased more than 3.5 times, the percentage of monocytes increased by 2 times. In the second experiment (animal blood), we observe a similar picture. The animals did not take any drugs, for the sake of the purity of the experiment. In the analysis of medicinal leeches in the second experiment in pathology, we observe an increased death of leeches by almost 4 times, the appearance of constrictions by 3 times, the intensity of blood spitting by more than 2 times (Tab. 1). When removing the eaten blood of sick animals from the stomach of a medicinal leech, we observe an increase in hematological and immune cells compared to conditionally healthy ones. The total number of erythrocytes increases by 21.4±2.9%, and leukocytes by 35.2±3.2%. In the blood leukocyte formula: a decrease in the percentage of lymphocytes by 60.5±4.5%, an increase in segmented neutrophils by 25.0±2.1%, an increase in the percentage of eosinophils by 2 times, an increase in band neutrophils by more than 11 times, which can testify to various pathological conditions in the body.

Discussion

It is known that in most cases of diseases, medicinal leeches are placed locally on the affected areas [1–3,6,9,11], and in various local pathological conditions, the total number of immune cells that try to cope and restore normal physiological conditions increases in these areas of the body. Therefore, it is possible that the medicinal leeches consumed a much larger number of immune cells, as a result of which, their immune system could not cope with the immune system of the host's blood and the leeches died, which we observe in our experiments. Since

these animals try to absorb more of the cellular composition of the blood than the liquid one during blood consumption, this is more likely. Also, the appearance of stretch marks on their body may be related precisely to the failure of their immune system, similar results were obtained by other scientists [23]. Secondly, in some cases, the mortality of leeches can be related to the toxicity of substances that a person could have taken before. For example, medicines or some narcotic drugs that are deadly to leeches. Therefore, to reject this negative factor, a second experiment was conducted. In which the blood of rats that did not take any substances was studied for the purity of the obtained experimental results. It should be noted that the percentage of death of medicinal leeches also increased when feeding with the blood of sick animals. This confirms our hypothesis obtained in the first experiment regarding the increase of immune cells in the area of pathology and the failure of the immune system of the ectoparasite to cope with the immune system of the host.

As a result of the research, we observed that medicinal leeches, when feeding on the blood of sick animals and people, began to die in most cases with deformed bodies, constrictions appeared on their bodies, and they began to spit blood more intensively. According to the data obtained, medicinal leeches and their blood eaten by the host can be used as a method of an express diagnostics in the early stages of the development of various pathologies of an inflammatory nature.

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References

[1] Baskova I.P. 2015. Hirudotheraphy scientific basements. Humoral link. Akvarius, Tula.

[2] Kumar S.A. 2012. Anti inflammatory effect of leech therapy in the patients of psoriasis (ek kustha). *Journal of Pharmaceutical and Scientific Innovation* 1(1): 71–74.

[3] Vallejo J.R., González J.A. 2015. The medicinal use of leeches in contemporary Spain: between science and tradition. *Acta Medico-Historica Adriatica* 13(1): 131–158.

[4] Huang H., Ruxue L., Li Y., Huang Q., Gao N., Zou W. 2021. Hirudo (Leech) for proliferative vitreous retinopathy: a protocol for systemic review and meta-analysis. *Medicine* 100(3): e24412. doi:10.1097/MD.0000000000024412

[5] Wollina U., Heinig B., Nowak A. 2016. Medicinal leech therapy (Hirudotherapy). *Our Dermatology Online* 7(1): 91–96. doi:10.7241/ourd.20161.24

[6] Liu J., Sui Y. 2015. Effects of leech superfine powder on the level of serum lipid in the rabbit of atherosclerosis model. *Journal of Liaoning University of Traditional Chinese Medicine* 17(6): 26–28.

[7] Rehman S. 2020. Management of diabetic foot ulcer by *Hirudo medicinalis* the “Healing Leech”. In: Diabetic foot ulcer. (Eds. M. Zubair, J. Ahmad, A. Malik, M.R. Talluri). Springer, Singapore: 315–330. doi:10.1007/978-981-15-7639-3_19

[8] Yang F., Li Y., Guo S., Pan Y., Yan C., Chen Z. 2021. *Hirudo* lyophilized powder ameliorates renal injury in diabetic rats by suppressing oxidative stress and inflammation. *Evidence-based Complementary and Alternative Medicine* 2021: article ID 6657673. doi:10.1155/2021/6657673

[9] Tashiro K., Fujiki M., Arikawa M., Kagaya Y., Miyamoto S. 2016. Free flap salvage after recurrent venous thrombosis by means of large-scale treatment with medicinal leeches. *Plastic and Reconstructive Surgery. Glob Open* 4(12): e1157. doi:10.1097/GOX.0000000000001157

[10] Krashenyuk A.I. 2020. Neurotrophic (neural stimulating) and neuromediator effects of *Hirudo medicinalis*. Pathogenetic mechanism of treatment of diseases of the nervous system of the human. *Acta Scientific Medical Sciences* 4(3): 4–9. doi:10.31080/ASMS.2020.04.0552

[11] Asgari S.A., Rostami S., Teimoori M. 2017. Leech therapy for treating priapism: case report. *Iranian Journal of Public Health* 46(7): 985–988.

[12] Baranzini N., De V.A., Orlandi V.T., Reguzzoni M., Monti L., de Eguileor M., Rosini E., Pollegioni L., Tettamanti G., Acquati F., Grimaldi A. 2020. Antimicrobial role of RNASET2 protein during innate immune response in the medicinal leech *Hirudo verbana*. *Frontiers in Immunology* 11: 1–18. doi:10.3389/fimmu.2020.00370

[13] Malik B., Astuti D.A., Arief D.J.F., Rahminiwati M. 2019. A study on antioxidative and antimicrobial activities of saliva extract of Indonesian local leeches. *IOP Conference Series*: *Earth and Environmental Science* 251: 1–16. doi:10.1088/1755-1315/251/1/012061

[14] Ojo P.O., Babayi H., Olayemi I.K., Peter O.O., Fadipe L.A., Baba E., Izebe K. 2018. Anti-tubercular activities and molecular characterization of salivary extract of leech (*Hirudo medicinalis*) against *Mycobacterium tuberculosis*. *Journal of Tuberculosis Research* 6(1): 1–9. doi:10.4236/jtr.2018.61001

[15] Shakouri A., Kahroba H., Hamishekar H., Abdolalizadeh J. 2021. Nanoencapsulation of *Hirudo*

medicinalis proteins in liposomes as a nanocarrier for inhibiting angiogenesis through targeting VEGFA in the Breast cancer cell line (MCF-7). *BioImpacts* 12(2): 115–126, doi:10.34172/bi.2021.39

[16] Kalender M.E., Comez G., Sevinc A., Dirier A., Camci C. 2010. Leech therapy for symptomatic relief of cancer pain. *Pain Medicine* 11(3): 443–445. doi:10.1111/j.1526-4637.2010.00800.x

[17] Fattakhov N.K., Tilyakhodzhaeva G.B., Abdulkhakimov A.R. 2021. Efficiency of application of hirudotherapy to have been having coronaviral infection. *Journal of Applied Research* 7(4): 2908–2911.

[18] Aminov R.F., Frolov A.K. 2018. The impact of fetal load of *Hirudo verbana* saline extract antigens morphometrical, hematological and immunological parameters of rats in the early stages of postembryonic development. *Annals of Parasitology* 64(1): 13–20. doi:10.17420/ap6401.127

[19] Aminov R., Aminova A. 2021. Indirect effect of substances of the hemophagous parasite *Hirudo verbana* on the immune system of the host rats. *Annals of Parasitology* 67(4): 603–610. doi:10.17420/ap6704.376

[20] Aminov R., Aminova A., Makyeyeva L. 2022. Morphological parameters of spleen and thymus of the male rats on the basis of the hirudological influence of *Hirudo verbena*. *Annals of Parasitology.*68(1): 55–60. doi:10.17420/ap6801.408

[21] Amani L., Motamed N., Mirabzadeh A.M., Dehghan S.M., Malek M. 2021. Semi-solid product of medicinal leech enhances wound healing in rats. *Jundishapur Journal of Natural Pharmaceutical Products* 16(4): e113910. doi:10.5812/jjnpp.113910

[22] Trenholme H.N., Masseau I., Reinero C.R. 2021. Hirudotherapy (medicinal leeches) for treatment of upper airway obstruction in a dog. *Journal of Veterinary Emergency and Critical Care* 31(5): 661–667. doi:10.1111/vec.13094

[23] Frolov A.K., Litvinenko R.A. 2015. Basic morphofunctional features of pharmaceutic leech (*Hirudo verbana* Carena, 1820) tissues in various forms of response after hirudotherapeutic procedures. *Annals of Parasitology* 61(1): 27–35.

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